A-Tract Induced DNA Bending is a Local Non-Electrostatic Effect

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The macroscopic curvature induced in double helical B-DNA by regularly repeated adenine tracts (A-tracts) is a long known, but still unexplained phenomenon. This effect plays a key role in DNA studies because it is unique in the amount and the variety of the available experimental information and, therefore, is likely to serve as a gate to the unknown general mechanisms of recognition and regulation of genome sequences. The dominating idea in the recent years was that, in general, macroscopic bends in DNA are caused by long range electrostatic repulsion between phosphate groups when some of them are neutralized by proximal external charges. In the case of A-tracts this may be specifically bound solvent counterions. Here we report about molecular dynamics simulations where a correct static curvature in a DNA fragment with phased adenine tracts emerges spontaneously in conditions where any role of counterions or long range electrostatic effects can be excluded.

RESULTS AND DISCUSSION

Although the macroscopic curvature of DNA induced by adenine-tracts (A-tracts) was discovered almost two decades ago[1,2] structural basis for this phenomenon remains unclear. A few models considered originally[3,4,5] suggested that it is caused by intrinsic conformational preferences of certain sequences, but all these and similar theories failed to explain experimental data obtained later.[6] Calculations show that the B-DNA duplex is mechanically anisotropic,[7] that bending towards minor grooves of some A-tracts is strongly facilitated,[8] and that the macroscopic curvature becomes energetically preferable once the characteristic A-tract structure is maintained by freezing or imposing constraints.[9,10,11] However, the static curvature never appears spontaneously in calculations unbiased *a priori* and these results leave all doors open for the possible physical origin of the effect. In the recent years the main attention has been shifted to specific interactions between DNA and solvent counterions that can bend the double helix by specifically neutralizing some phosphate groups.[12,13,14,15,16] The possibility of such mechanism is often evident in protein-DNA complexes, and it has also been demonstrated by direct chemical modification of a duplex DNA.[14] In the case of the free DNA in solution, however, the available experimental observations are controversial.[16,17] Molecular dynamics simulations of a B-DNA in an explicit counterion shell could neither confirm nor disprove this hypothesis.[18] Here we report the first example where stable static curvature emerges spontaneously in molecular dynamics simulations. Its direction is in striking agreement with expectations based upon experimental data. However, we use a minimal B-DNA model without counterions, which strongly suggests that they hardly play a key role in this effect.

Figure 1 exhibits results of a 10 ns simulation of dynamics of a 25-mer B-DNA fragment including three A-tracts separated by one helical turn. This sequence has been constructed after many preliminary tests with shorter sequence motives. Our general strategy came out from the following considerations. Although the A-tract sequences that induce the strongest bends are known from experiments, probably not all of them would work in simulations. There are natural limitations, such as the precision of the model, and, in addition, the limited duration of trajectories may be insufficient for some A-tracts to adopt their specific conformation. Also, we can study only short DNA fragments, therefore, it is preferable to place A-tracts at both ends in order to maximize the possible bend. There is, however, little experimental evidence of static curvature in short DNA fragments, and one may well expect the specific A-tract structure to be unstable near the ends. That is why we did not simply take the strongest experimental "benders", but looked for sequence motives that in calculations readily adopt the characteristic local structure, with a narrow minor groove profile and high propeller twist, both in the middle and near the ends of the duplex. The complementary duplex AAAATAGGCTATTTTAGGCTATTTT has been constructed by repeating and inverting one such motive.

The upper trace in plate (a) shows the time dependence of rmsd from the canonical B-DNA model. It fluctuates below 4 Å sometimes falling down to 2 Å, which is very low for the double helix of this length indicating that all helical parameters are well within the range of the B-DNA family. The lower surface plot shows the time evolution of the minor DNA groove. The surface is formed by 75 ps time-averaged successive minor groove profiles, with that on the front face corresponding to the final DNA conformation. The groove width is evaluated by using space traces of C5' atoms as described elsewhere[19]. Its value is given in angströms and the corresponding canonical B-DNA level of 7.7 Å is marked by the straight dotted lines on the faces of the box. It is seen that the

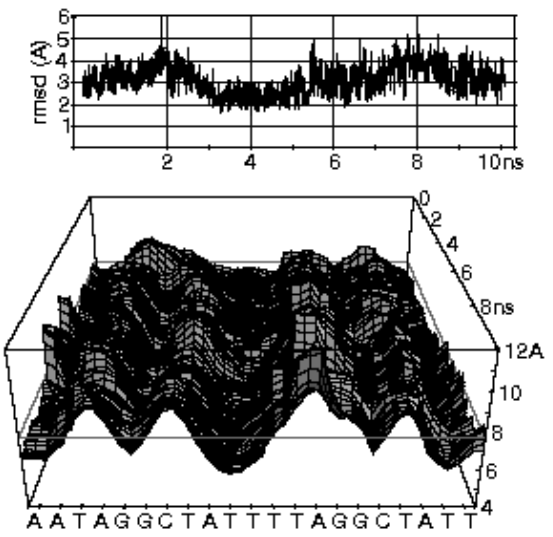

FIG. 1. (a)

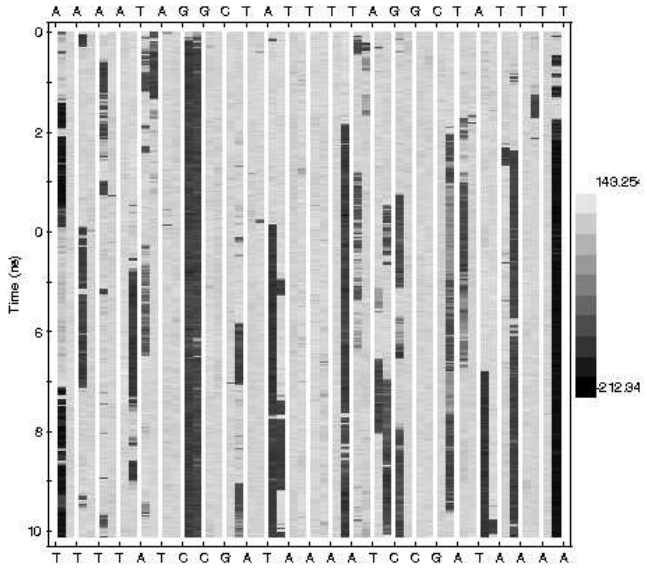

FIG. 1. (b)

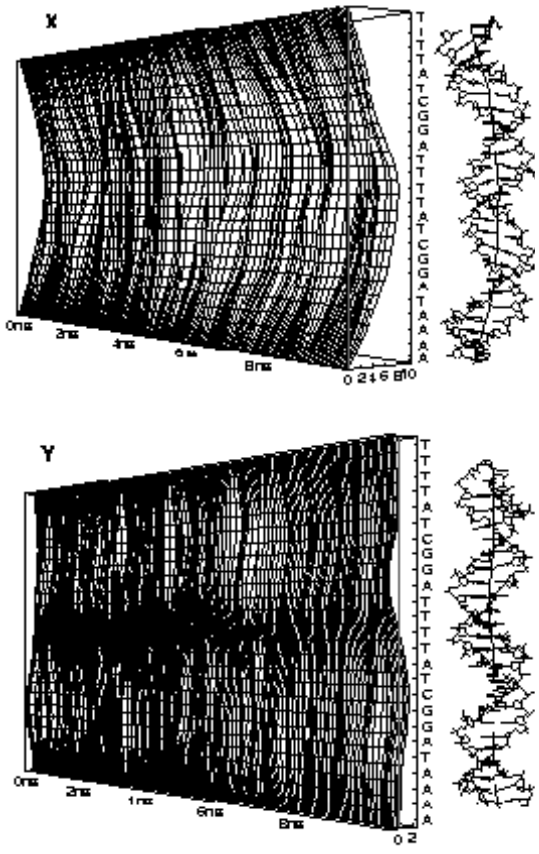

FIG. 1. (c)

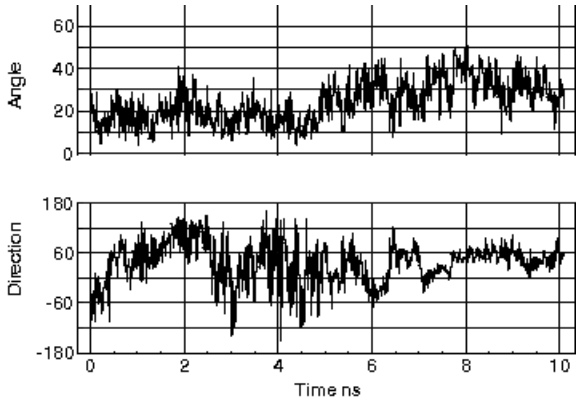

FIG. 1. Representative results from the first 10 ns MD simulation of a 25-mer double helical DNA fragment. (a) The time variation of the heavy atom rmsd from the canonical B-DNA form and the evolution of the profile of the minor groove. (b) Dynamics of $B_I \leftrightarrow B_{II}$ backbone transitions. (c) The time evolution of the optimal helical axis, which is a best fit axis of coaxial cylindrical surfaces passing through sugar atoms. In all cases considered here it is very close to that produced by the Curves algorithm.[30] Two perpendicular projections are shown with the corresponding views of the average conformation during the last nanosecond shown on the right. (d) The time variation the bending angle and direction. The bending angle is measured between the two ends of the optimal helical axis. The bending direction is characterized by the angle between the X-projection plane in plate (c) and the xz plane of the local DNA coordinate frame constructed in the center of the duplex according to the Cambridge convention[20].

overall groove shape has established after 2 ns and remained stable later, with noticeable local fluctuations. In all A-tracts the groove strongly narrows towards 3' ends and widens significantly at the boundaries. There are two less significant relative narrowings inside non A-tract sequences as well.

Dynamics of $B_I \leftrightarrow B_{II}$ backbone transitions are shown in plate (b). The B_I and B_{II} conformations are distinguished by the values of two consecutive backbone torsions, ε and ζ. In a transition they change concertedly from (t,g^-) to (g^-,t). The difference $\zeta - \varepsilon$ is, therefore, positive in B_I state and negative in B_{II}, and it is used in Fig. (d) as a monitoring indicator, with the corresponding gray scale levels shown on the right. Each base pair step is characterized by a column consisting of two sub-columns, with the left sub-columns referring to the sequence written at the top in 5'-3' direction from left to right. The right sub-columns refer to the complementary sequence shown at the bottom. It is seen that, in A-tracts, the B_{II} conformation is preferably found in ApA steps and that $B_I \leftrightarrow B_{II}$ transitions in neighboring steps often occur concertedly so that along a single A-strand B_I and B_{II} conformations tend to alternate. The pattern of these transitions reveals rather slow dynamics and suggests that MD trajectories in the 10 ns time scale are still not long enough to sample all relevant conformations. Note, for instance, a very stable B_{II} conformation in both strands at one of the GpG steps.

Plate (c) shows the time evolution of the overall shape of the helical axis. The optimal curved axes of all DNA conformations saved during dynamics were rotated with the two ends fixed at the OZ axis to put the middle point at the OX axis. The axis is next characterized by two perpendicular projections labeled X and Y. Any time section of the surfaces shown in the figure gives the corresponding axis projection averaged over a time window of 75 ps. The horizontal deviation is given in angströms and, for clarity, its relative scale is two times increased with respect to the true DNA length. Shown on the right are two perpendicular views of the last one-nanosecond-average conformation. Its orientation is chosen to correspond approximately that of the helical axis in the surface plots.

It is seen that the molecule maintained a planar bent shape during a substantial part of the trajectory, and that at the end the bending plane was passing through the three A-tracts. The X-surface clearly shows an increase in bending during the second half of the trajectory. In the perpendicular Y-projection the helical axis is locally wound, but straight on average. The fluctuating pattern in Y-projection sometimes reveals two local maxima between A-tracts, which corresponds to two independent bends with slightly divergent directions. One may note also that there were at least two relatively long periods when the axis was almost straight, namely, around 3 ns and during the fifth nanosecond. At the same time, straightening of only one of the two bending points is a more frequent event observed several times in the surface plots.

Finally, plate (d) shows the time fluctuations of the bending direction and angle. The bending direction is characterized by the angle between the X-projection plane in plate (c) and the xz plane of the local DNA coordinate frame constructed in the center of the duplex. According to the Cambridge convention[20] the local x direction points to the major DNA groove along the short axis of the base-pair, while the local z axis direction is adjacent to the optimal helicoidal axis. Thus, a zero angle between the two planes corresponds to the overall bend to the minor groove exactly at the central base pair. In both plots, short time scale fluctuations are smoothed by averaging with a window of 15 ps. The total angle measured between the opposite axis ends fluctuates around 10-15° in the least bent states and raises to average 40-50° during periods of strong bending. The maximal instantaneous bend of 58° was observed at around 8 ns.

The bending direction was much more stable during the last few nanoseconds, however, it fluctuated at a roughly constant value of 50°starting from the second nanosecond. This value means that the center of the observed planar bend is shifted by approximately two steps from the middle base pair so that its preferred direction is to the minor groove at the two ATT triplets, which is well distinguished in plate (c) as well, and corresponds to the local minima in the minor groove profiles in plate (a). During the periods when the molecule straightened the bending direction strongly fluctuates. This effect is due to the fact that when the axis becomes straight the bending plane is not defined, which in our case appears when the central point of the curved axis passes close to the line between its ends. It is very interesting, however, that after the straightening, the bending is resumed in approximately the same direction.

Figure 2 exhibits similar data for another 10 ns trajectory of the same DNA fragment, computed in order to check reproducibility of the results. A straight DNA conformation was taken from the initial phase of the previous trajectory, energy minimized, and restarted with random initial velocities. It shows surprisingly similar results as regards the bending direction and dynamics in spite of a somewhat different minor groove profile and significantly different distribution of B_I and B_{II} conformers along the backbone. Note that in this case the helical axis was initially S-shaped in X-projection, with one of the A-tracts exhibiting a completely opposite bending direction. Fluctuations of the bending direction are reduced and are similar to the final part of the first trajectory, which apparently results from the additional re-equilibration. In this case the maximal instantaneous bend of 71° was observed at around 4 ns.

Comparison of traces in plates (a) and (d) in Figs. 1 and 2 clearly shows that large scale slow fluctuations of rmsd are caused by bending. The rmsd drops down to 2 Å when the duplex is straight and raises beyond 6 Å in strongly bent conformations. In both trajectories the molecule experienced many temporary transitions to straight conformations which usually are very short liv-

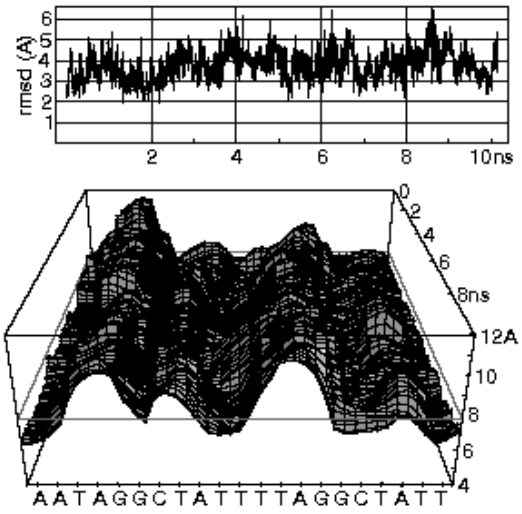

FIG. 2. (a)

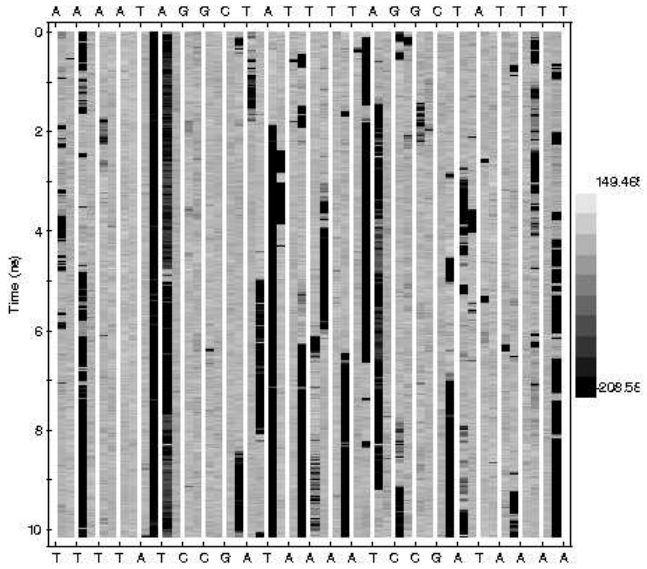

FIG. 2. (b)

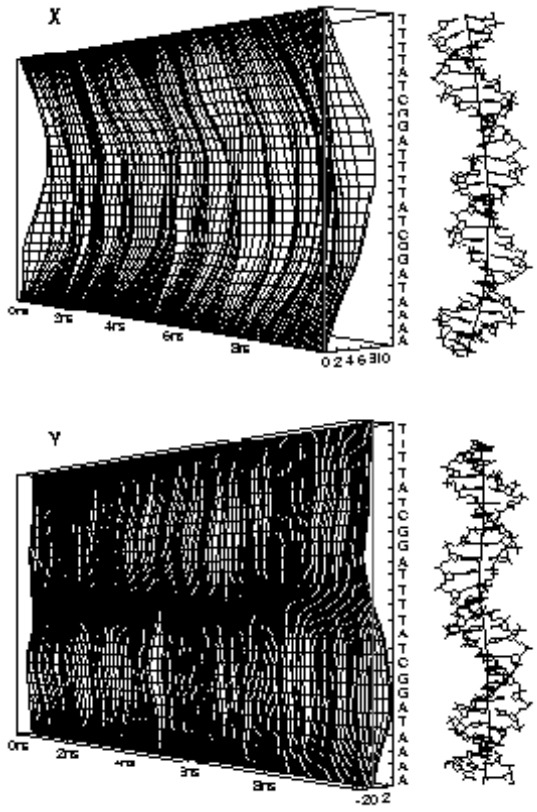

FIG. 2. (c)

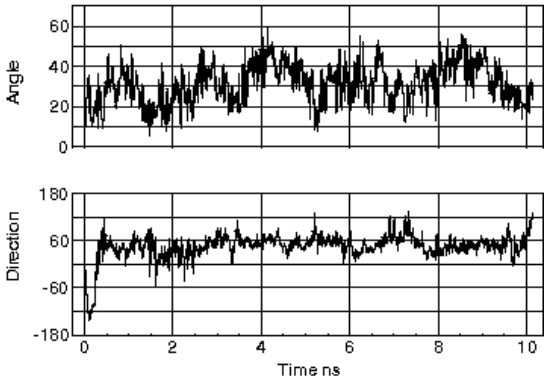

FIG. 2. Representative results from the second 10 ns MD trajectory of the same DNA fragment. Notation as in Fig. 1.

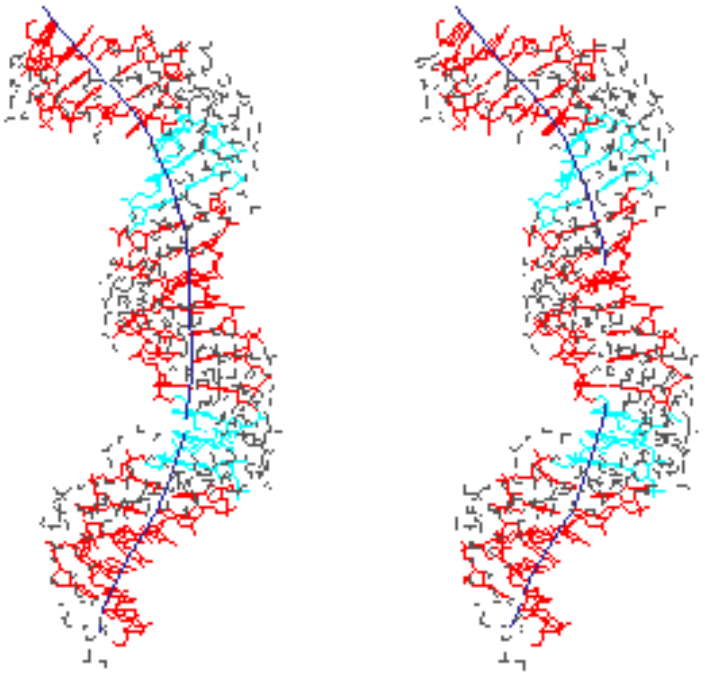

FIG. 3. A stereo snapshot of the system at around 8.5 ns of the second trajectory. AT base pairs are shown in red and GC base pairs in blue.

ing. These observations suggest that the bent state is relatively more stable than the straight one and, therefore, the observed behavior corresponds to static curvature. In conformations averaged over successive one nanosecond intervals the overall bending angle is 35-45° except for a few periods in the first trajectory. Figure 3 shows a snapshot from around 8.5 ns of the second trajectory where the rmsd from the straight canonical B-DNA reached its maximum of 6.5 Å. The strong smooth bent towards the minor grooves of the three A-tracts is evident, with the overall bending angle around 61°.

All transformations exhibited in Figs. 1 and 2 are isoenergetic, with the total energy fluctuating around the same level established during the first nanosecond already, and the same is true for the average helicoidal parameters. Plates (b), however, indicate that there are much slower motions in the system, and this observation precludes any conclusions concerning the global stability of the observed conformations. Moreover, we have computed yet another trajectory for the same molecule starting from the canonical A-DNA form. During 10 ns it converged to a similarly good B-DNA structure with the same average total energy, but the bending pattern was not reproduced. It appears, therefore, that the conformational space is divided into distinct domains, with transitions between them probably occurring in much longer time scales. However, the very fact that the stable curvature in good agreement with experimental data emerges in trajectories starting from a featureless straight canonical B-DNA conformation strongly suggests that the true molecular mechanism of the A-tract induced bending is reproduced. Therefore, it cannot depend upon the components discarded in our calculations, notably, specific interactions with solvent counterions and long-range electrostatic effects.

We are not yet ready to present a detailed molecular mechanism responsible for the observed curvature because even in this relatively small system it is difficult to distinguish the cause and the consequences. We believe, however, that all sorts of bending of the double helical DNA, including that produced by ligands and that due to intrinsic sequence effects, have its limited, but high flexibility as a common origin. Its own conformational energy has the global minimum in a straight form, but this minimum is very broad and flat, and DNA responds by distinguishable bending to even small perturbations. The results reported here prove that in the case of A-tracts these perturbations are produced by DNA-water interactions in the minor groove. Neither long range phosphate repulsion nor counterions are essential. The curvature is certainly connected with the specific A-tract structure and modulations of the minor groove width, but it does not seem to be strictly bound to them. In dynamics, conformations, both smoothly bent and kinked at the two insertions between the A-tracts, are observed periodically. Note also, that the minor groove profile somewhat differs between the two trajectories and that it does not change when the molecule straightens. We strongly believe, however, the experimental data already available will finally allow one to solve this problem by theoretical means, including the approach described here, and we continue these attempts.

METHODS

Molecular dynamics simulations have been performed with the internal coordinate method (ICMD)[21,22] including special technique for flexible sugar rings[23]. The so-called "minimal B-DNA" model was used[24,25] which consists of a double helix with the minor groove filled with explicit water. Unlike the more widely used models, it does not involve explicit counterions and damps long range electrostatic interactions in a semi-empirical way by using distance scaling of the electrostatic constant and reduction of phosphate charges. The DNA model was same as in earlier reports,[24,25] namely, all torsions were free as well as bond angles centered at sugar atoms, while other bonds and angles were fixed, and the bases held rigid. AMBER94[26,27] force field and atom parameters were used with TIP3P water[28] and no cut off schemes. With a time step of 10 fs, these simulation conditions require around 75 hours of cpu per nanosecond on a Pentium II-200 microprocessor.

The initial conformations were prepared by vacuum en-

ergy minimization starting from the fiber B-DNA model constructed from the published atom coordinates.[29] The subsequent hydration protocol to fill up the minor groove[24] normally adds around 16 water molecules per base pair. The heating and equilibration protocols were same as before[24,25]. During the runs, after every 200 ps, water positions were checked in order to identify those penetrating into the major groove and those completely separated. These molecules, if found, were removed and next re-introduced in simulations by putting them with zero velocities at random positions around the hydrated duplex, so that they could readily re-join the core system. This procedure assures stable conditions, notably, a constant number of molecules in the minor groove hydration cloud and the absence of water in the major groove, which is necessary for fast sampling[25]. The interval of 200 ps between the checks is small enough to assure that on average less then one molecule is repositioned and, therefore, the perturbation introduced is considered negligible.

ACKNOWLEDGEMENTS

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APPENDIX

This section contains comments from anonymous referees of peer-review journals where the manuscript has been considered for publication, but rejected.

A. Journal of Molecular Biology

1. First referee

Dr. Mazur describes molecular dynamics simulations where a correct static curvature of DNA with phased A-tracts emerges spontaneously in conditions where any role of counterions or long range electrostatic effects can be excluded.

I have several problems with this manuscript:

1) The observed curvature is dependent on the starting model. In fact the manuscript uses the phrase 'stable static curvature' incorrectly to describe what is probably a trapped metastable state. The observed curve is neither stable nor static.

2) The choice of DNA sequence seems to be biased toward that which gives an altered structure in simulations, ad is not that which gives the most pronounced bend in solution. I would suggest a comparison of (CAAAATTTTTG)n and (CTTTTAAAAG)n.

3) The result is not consistent with solution results. See for example:

Prodin, F., Cocchione, S., Savino, M., & Tuffillaro, A. "Different Interactions of Spermine With a Curved and a Normal DNA Duplex - (Ca(4)T(4)G)(N) and (Ct(4)a(4)G)(N) - Gel -Electrophoresis and Circular-Dichroism Studies" (1992) Biochemistry International 27, 291-901.

Brukner, l, Sucis, S., Dlakic, M., Savic, A., & Pongor, S. "Physiological concentrations of magnesium ions induces a strong macroscopic curvature in GGGCCC - containing DNA" (1994) J. Mol. Biol. 236, 26-32.

Diekmann, S., & Wang, J. C. "On the sequence determinants and flexibility of the kinetoplast DNA fragment with abnormal gel electrophoretic mobilities" (1985) J. Mol. Biol. 186, 1-11.

Llaudnon, C. H., & Griffith, J. D. "Cationic metals promote sequence-directed DNA bending" (1987) Biochemistry 26, 3759-3762.

4) The result is not consistent with other simulations. See for example:

Feig, M., & Pettitt, B. M. "Sodium and Chlorine ions as part of the DNA solvation shell" (1999) Biophys. J. 77, 1769-81.

5) The results should be given by objective statistical descriptions rather than a series of spot examples, as in "sometimes reveals two independent bends".

2. Second referee

This manuscript describes the modeling of a 25-residue DNA duplex using molecular dynamics simulations. The DNA sequence in question contains 3 A/T tracts arranged in-phase with the helix screw and thus is expected to manifest intrinsic bending. Unlike previous MD studies of intrinsically bent DNA sequences, these calculations omit explicit consideration of the role of counterions. Because recent crystallographic studies of A-tract-like DNA sequence have attributed intrinsic bending to the localization of counterions in the minor groove, the present finding that intrinsic bending occurs in the absence of explicit counterions is important for understanding the underlying basis of A-tract-dependent bending.

Overall, the MD procedure appears sound and the calculations were carried out with obvious care and attention to detail. There are two specific issues raised by this study that should be addressed in revision, however.

1. Although the sequence chosen for this study was based on a canonical, intrinsically-bent motif consisting of three A tracts, it is unclear to what extent intrinsic bending has been experimentally shown for this particular sequence. There are known sequence-context effects that modulate A-tract-dependent bending and thus the author should refer the reader to data in the literature or show experimentally that intrinsic bending of the expected magnitude occurs for this particular sequence. Moreover, one A tract is out-of-phase with respect to the others and it is therefore not clear how this contributes to the overall bend. The author is understandably concerned about end effect with short sequences; this problem can be ameliorated by examining DNA fragments that constrain multiple copies of the chosen motif or by extending the ends of the motif with mixed-sequence DNA.

2. Notwithstanding the authors remark bout separating the cause and the effects with respect to intrinsic bending some comments about the underlying mechanism of bending seem appropriate. It would be particularly useful to know whether average values of any specific conformational variables are unusual or whether strongly bent states are consistent with narrowing of the minor groove within A-tracts, for example.